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## Trigona Honey, a Natural Bee Product Promotes mRNA Foxp3 Expression in Healthy in Mice Balb/c Strain

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### Abstract

Transcription factor, *Foxp3* Treg plays an important role in the balance of the immune system, diets containing polyphenols and flavonoids could increase the expression of *FOXP3* mRNA although some studies have contrary results. Trigona honey is a specific honey from Trigona bees containing polyphenols and could influence the immune homeostasis. There have never been studies proving its effect on the expression of *Foxp3* mRNA as the transcription factor of Regulatory T Cell. It was a laboratory research; mice Balb/c were divided into the reference, positive and treatment groups. The reference group was only given standard feed and positive control was intraperitoneally injected with *Salmonella Enterica serovar Typhi*. The treatment group was divided into 2 groups and given Trigona honey using canule with both doses of 0.23 ml/20 g Bw and 0.27 ml/20 g Bw daily for 10 days respectively. *Foxp3* mRNA expression was examined by real-time RT-PCR. Repeated Anova and One Way Anova were used as the statistical methods, a *p*-value of less than 0.050 at the final analysis was considered indicating statistical significance. Results indicated *Foxp3* mRNA expression of the groups given by honey was higher than the control group.

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The highest *Foxp3* mRNA expression in Trigona honey was the group given with a dose of 0.27 ml/20 g Bw ( $p=0.000$ , however, the group given Trigona Honey with a dose of 0.23 ml/20 g Bw also had moderate *Foxp3* mRNA expression. These data suggested that Trigona honey could induce *Foxp3* mRNA expression. The higher dose given, the higher *Foxp3* mRNA expression.

**Keywords:** Trigona; Honey; *Foxp3*; *Salmonella typhi*.

## 1. Introduction

Regulatory T cells (Treg) have pivotal roles in immune homeostasis, protein *Foxp3* as both marker and transcription factor of Treg cell needs stable expression to maintain normal function of Treg. [1,2] . Some diseases with immunological self-tolerance disorders, such as rheumatoid arthritis, multiple sclerosis, arthritis and encephalomyelitis have close links with imbalance function of *Foxp3* Treg. Results of the advanced studies showing that *Salmonella* have been attenuated by making a vaccine for severe cases of arthritis and encephalomyelitis that indicate its capability to induce *Foxp3* regulatory T cells. Despite its success in inducing *Foxp3* Treg, the use of pathogenic bacteria are still being debated [3-5]. Bacterial infections as *Salmonella leishmania* and *Mycobacterium tuberculosis* also need the balance of *Foxp3* expression to control homeostasis between immunosuppressive and immunopathology [6-11].

Potency of natural products to induce T Reg cells continue to be studied and also their important compounds contributed to the induction of *Foxp3* expression. Several studies have found that polyphenols could induce Treg cells through the expression of *Foxp3* transcription factors [12]. Nevertheless, some studies have shown polyphenols and flavonoids actually inhibited Treg activity and decreased the expression of *Foxp3* [13, 14].

Trigona honey contains polyphenols and flavonoids compounds and it is always available and use daily as a natural product, product of Trigona bees from Masamba, South Sulawesi contained total phenols and quercetin [15-17]. Compounds of Honey has various roles as both antibacterial and anti-inflammatory and could affected cytokines interleukin 6 (IL-6) and interleukin-10 (IL-10) [18]. This study aimed to analyze the effect of Trigona honey from Masamba district, South Sulawesi Province, Indonesia to the increase of *Foxp3* mRNA expression and to test whether there are different conditions among healthy mice subjects based on doses given.

## 2. Material and Methods

Study was conducted at Microbiology Laboratory, Immunology and Biomolecular Laboratory and Biopharmacology Laboratory of Hasanuddin University. Ethical procedure has been approved by Ethical Commission of Hasanuddin University. Samples were 20 mice BALB/c and categorized into four groups, where each group consisted of 5 mice. Trigona honey was compiled from Masamba, a district in South Sulawesi Province of Indonesia. In Indonesia, besides Kalimantan Island, one of the well-known regions as the producer of Trigona Honey is Masamba district, located in South Sulawesi Province [19].

### 2.1 Materials

Samples of honey were taken from Masamba, North Luwu District, South Sulawesi Province, Indonesia. Afterward, samples were precipitated in dark period for 72 hours in hygienic condition, and then they were stored into a heating/drying oven to decrease their water contents.

## 2.2 Experimental animals

Male mice Balb/c with 25-27 gr were kept in individual cages under standard condition (12-h light and 12-h dark condition). They were given water and chow diet *ad libitum* after 7 days adaptation to environmental condition. The experimental protocol was approved by Ethical use of Animal in Ethical Commission of Hasanuddin University.

## 2.3 Honey intervention

Male Mice Balb/c were divided into four groups (each group consisted of 5 mice) and received different intervention, the first group as the reference group was not given any intervention, but only standard diet. The second group was positive control, induced intraperitoneally by *Salmonella typhi*  $10^3$ , group 2 and 3 were given honey with both 0.23 ml/20 g Bw and 0.27 ml/20 g Bw for 10 days respectively.

## 2.4 RNA isolation and real-time RT-PCR

Blood samples were taken in the tail, total mRNA was isolated from blood samples obtained using the Boom protocol methods. Quantitative *real-time polymerase chain reaction* used BRILLIANT II SYBR® by following product instructions. Primary *Foxp3* synthesized using Macrogen (Korea), Primer sequences was FW-TTTACTCGCATGTTCGCCTACTT, RV-TCAAATTCATCTACGGTCCACAC [12, 20].

## 2.5 Statistical analysis

Data were presented with figures and tables and expressed as means and standard deviation (SD). Statistical test was one- way ANOVA. All *p*-values  $\leq 0.05$  were considered significant.

## 3. Results

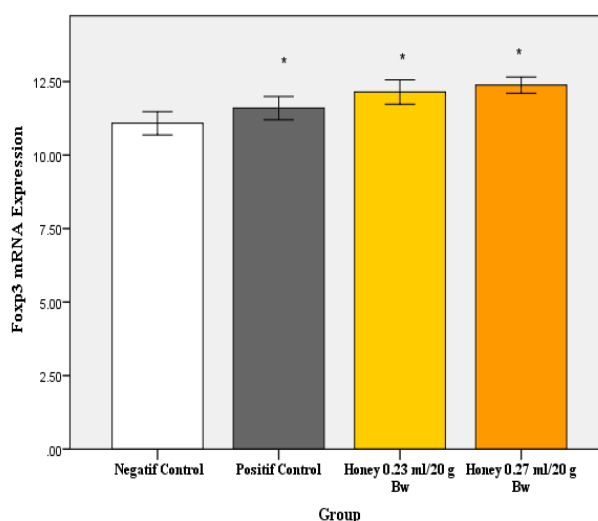
Data showed the increase of *Foxp3* mRNA expression in healthy mice treated by honey, the higher dose of honey, the higher *Foxp3* mRNA expression. Healthy mice induced by *Salmonella typhi* also showed the increase of *Foxp3* mRNA expression, but still lower than the groups treated by honey (Figure1 and Table 1).

The increase of *Foxp3* mRNA expression of healthy mice given Trigona Honey was significance compared to the control groups, both negative and positive controls and all groups significantly difference (Figure1 and Table 1).

**Table 1:** The *Foxp3* mRNA Expression of Healthy Mice and Control Groups

Group	<i>Foxp3</i> mRNA Expression (Mean±SD)	<i>p</i> *
Negative Control	11.04±0.13	0.000 <sup>a</sup>
Positive Control	11.07 ±0.24	
Honey 0.23 ml/20 gBw	12.14±0.20	
Honey 0.27 ml/20 gBw	12.37±0.13	

\* One-way ANOVA Statistical Test

<sup>a</sup> Significant ( $P \leq 0.05$ )

**Figure 1:** Comparison of mean values for the *Foxp3* mRNA Expression among groups given both Trigona Honey and Control groups. Mice Balb/c were divided into 4 groups (n=5), one group as the reference and one group as the positive control (Induced Salmonella), 2 groups were given Trigona honey for 10 days with different doses. \*Values are significantly different from the control group at  $p \leq 0.05$ .

#### 4. Discussion

Polyphenols (gallic acid) and flavonoid (quercetin) compounds of Trigona Honey are higher than Trigona Carbonaria derived from Australia [15]. In this study, the data suggested that honey could increase *Foxp3* mRNA expression, the higher dose given, the higher *Foxp3* expression. *Salmonella* induction also enhanced the expression of FOXP3, but still much lower than honey.

One of the theories underlying the mechanism of polyphenols affecting the immune system is the epigenetic mechanism, such as DNA methylation [21]. DNA methylation is controlled by DNA methyltransferase (DNMT)

[22]. *Foxp3* expression epigenetically regulated by DNA methylation, a diet suggested containing polyphenols as a DNA methyltransferase inhibitor (DNMT) that enhances demethylation and can induce the expression of *Foxp3* [23,24, 25].

Other mechanisms are regarded play important roles through cytokine transforming growth factor  $\beta$  (TGF- $\beta$ ) because this cytokine is considered one of the factors which necessary in both initiating and maintaining the expression of *Foxp3*. Flavonoids, quercetin may affect the alteration of TGF- $\beta$  and although research is still lacking, results of some studies indicate that honey can increase TGF- $\beta$  [26-29]. Increased systemic TGF- $\beta$  will raise the frequency of TREM, a mechanism of TGF- $\beta$  triggering the FOXP3 gene expression that involves the induction of Smad3 (pSmad3). Induced Smad3, initially binds to the enhancer site of *Foxp3* in intron 2 and interacts with nuclear factor-kB, NFATc2 and CREB that binds with *Foxp3* promoter [30].

Results of this study indicated that honey could increase the expression of *Foxp3 mRNA*, but did not examine the mechanisms involved in the process, so it could be investigated in future studies. It is also interesting to search the benefits of honey in auto-immune diseases considering it has been proven able to induce the expression of *Foxp3 mRNA*.

## 5. Conclusion

In this study, the data showed that honey could increase *Foxp3 mRNA* expression; the higher the dose given, the higher *Foxp3 mRNA* expression. Induction of *Salmonella* also enhanced the expression of *Foxp3*, but still much lower than honey.

## Competing interest

The authors declare that they have no competing interests.

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